85. The cultured cell of claim 81, the genome of which is genetically altered by adding, modifying, substituting, or deleting one or more genes.

86. The cultured cell of claim 85, the genome of which is genetically altered by addition, modification, substitution, or deletion of one or more genes that encode an enzyme, a growth factor, or a cytokine.

87. The cultured cell of claim 81, the genome of which is genetically altered by a method comprising homologous recombination.

REMARKS

This Reply is responsive to the Office Action dated December 19, 2001. Entry of the foregoing and reconsideration on the merits pursuant to 37 CFR 1.112 is respectfully requested. The application is amended as set forth above. In accordance with the rules for amending applications set forth in 37 CFR 1.121, the attached appendix provides copies of the amended paragraphs and claims that are marked-up to show the changes that are made.

Support for the Amendments of the Specification:

Paragraphs beginning on pages 19 and 22 are amended to correct typographic errors.

The paragraph beginning on page 5 that describes a study of cross-species nuclear transfer by Wolfe et al. (*Theriogenology*, 33:350 (1990)) is amended to avoid incorrectly implying that Wolfe et al. obtained a cross-species blastocyst only when a bovine nucleus was transferred into a buffalo nucleus. Wolfe et al. reported that in addition to the blastocyst obtained from the bovine/ buffalo NT unit, four of the NT units that they obtained by transferring bovine nuclei into goat oocytes cleaved to produce multicellular cross-species

embryos, and that one of these progressed to form a "morphologically normal blastocyst" (see the text under the table).

No new matter is added by the above amendments.

Support for the Amendment of the Claims:

Original claims 1-35 are canceled and new claims 36-87 are submitted. The new claims are limited to subject matter encompassed by 1-35 that were examined and addressed in the Office Action dated December 19, 2001.

New claims 38-87 are directed to methods that use differentiated mammalian donor cells and mammalian recipient oocytes, and to cells produced by the disclosed methods which are not themselves embryos. Claims directed to pluripotent embryonic stem cells and to differentiated cells derived from these are withdrawn, and will be re-submitted in a different application. The specification expressly states that an object of the invention is to provide a novel method for producing embryonic cells which involves cross-species nuclear transfer (page 7, lines 27-31), and teaches that cross-species embryonic cells produced by the claimed methods are useful, for example, in studying cell differentiation and the effects of expression of specific genes on early development (e.g., see page 9, lines 4-6; and page 28, lines 29-32).

New independent claim 36, and dependent claims 37-71 are directed to a method comprising transferring the genome of a differentiated donor cell of one mammalian species into a recipient oocyte of a different mammalian species to produce an activated nuclear transfer unit, i.e., an embryo, that is capable of dividing to form a multicellular structure, e.g., a blastocyst, and to isolating and culturing embryonic cells from the resulting multicellular structure. Support for these claims is found, for example, at page 12, lines 16-22, and in original claims 1-14 and 17-23.

New dependent claims 72-87 are directed to a method wherein the genome of a cell derived from a nuclear transfer embryo produced according to the claimed invention is genetically altered, e.g., by addition, modification, substitution, or deletion of one or more genes. Support for these claims is found, for example, in the paragraph bridging pages 26-27.

Support for the use of a differentiated somatic donor cell from an adult human as recited in new claims 46 and 54 is found, for example, in Example 1.

Support for the method wherein the differentiated donor cell is a germ cell or a somatic cell, as recited in claims 41 and 42 is found, for example, at page 14, lines 13-14.

Support for the method wherein the differentiated donor cell is a human or non-human cell of the type recited in claims 43-47 is found, for example, in the paragraph bridging pages 13-14.

Support for the method wherein the differentiated donor cell is from an ungulate as recited in claim 48 is found, for example, at page 12, lines 22-25.

Support for the method wherein the oocyte is from one of the mammals recited in claims 49-53 is found, for example, at page 14, lines 21-27.

Support for the method wherein the differentiated donor cell is a human cell and the oocyte is a bovine oocyte as recited in claim 54 is found, for example, in the paragraph bridging pages 11-12.

Support for the method wherein the activated nuclear transfer unit is cultured on a feeder layer of fibroblast cells to produce a multicellular structure as recited in claim 55 is found, for example, at page 21, lines 19-23.

Support for the method wherein an embryonic cell is isolated from the multicellular structure as recited in claim 56 is found, for example, in the paragraph bridging pages 21-22.

Support for the method wherein an embryonic cell is isolated from a multicellular structure of about 2 to 400 cells as recited in claim 57 is found, for example, at lines 2-3 of page 22.

Support for the method in which the activated nuclear transfer unit is cultured to produce a blastocyst from which embryonic cells are isolated, and cell lines are produced from the isolated embryonic cells, as recited in claims 58-60, is found, for example, in Example 1, which discloses producing such a line of cells having a human genome and bovine mitochondria.

The new claims do not contain new matter.

Regarding Rejection of the Claims Under 35 U.S.C. §101:

Claims 1–35 were rejected under 35 U.S.C. §101 as being directed to non-statutory subject matter because the claims may be construed to read on a human embryo. Claims 18-25 were further rejected under 35 U.S.C. §101 as being drawn to natural products, because the claimed embryonic or stem-like cells are not distinguished from naturally-produced embryonic or stem cells.

Claims 1–35 are canceled. New claims 36-60 and 72-74 recite methods, and so cannot be construed to read on a human being. New claims 61-71 and 75-87 correspond to canceled claims 18-23 in being drawn to isolated embryonic cells having genomic DNA of one mammalian species and mitochondria of a different mammalian species, or in reciting cultured cells derived from such cells. New claims 61-71 and 75-87 specifically recite that they are directed to a cell that is not itself an embryo or part of an embryo, or that they are derived from such cells. The new claims do not encompass a human zygote or other embryonic cells that cleave and proceed to develop into a human being, nor do they read on

cells that are part of a human being. Accordingly, the Applicants respectfully request withdrawal of the rejection of claims under 35 U.S.C. §101 as being directed to non-statutory subject matter.

The Applicants respectfully traverse the rejection of the claimed cells under 35 U.S.C. §101 as being natural products. As described in the specification and as recited in the claims, the claimed cells produced by cross-species nuclear transfer according to the claimed invention are distinct from natural cells in having genomic DNA of one species and mitochondria of another species. Such cells are useful for studying the differentiation of embryonic cells (as described, for example, on page 28, lines 29-32, of the specification) in ways that are not possible with cells having genomic DNA and cytoplasm of the same species.

Differences between the developmental/differentiation potential of embryonic cells produced by cross-species nuclear transfer and embryonic cells having nuclei and cytoplasm of the same species are evident in the experimental results reported by Dominko et al. and First et al. Dominko et al. (Biology of Reproduction 60:1496-1502, 1999, copy attached) transferred differentiated donor cells (fibroblasts) from cows, sheep, pigs, monkeys, and rats into enucleated bovine oocytes and studied the subsequent development of the resulting nuclear transfer (NT) units, in order to study cell cycle compatibilities between genomes of donor cells and cytoplasm of recipient oocytes after nuclear transfer. They reported that blastocysts were generated from NT units produced by transfer of nuclei of cows, sheep, pigs, and monkeys into enucleated bovine oocytes, but not by transfer of rat nuclei into bovine oocytes (Table 1, page 1499). They observed that the timing of the first two mitotic divisions of the cross-species NT units appeared to be under control of the bovine oocyte, but that timing of subsequent divisions and of the onset of the blastocoel cavity appeared to be under genomic control and reflected the timing of these events of the species of the nuclear donor

cell (see p. 1498, right column). They also reported that blastocysts generated from the cross-species NT units failed to develop into fetuses after being transferred to surrogate female animals (see p. 1499, left column). As discussed by Dominko et al., in order for the genome of a differentiated donor cell to direct development of an embryo formed by nuclear transfer into an enucleated oocyte, the donor chromatin must be "re-programmed" to an embryonic state by factors present in the oocyte cytoplasm. While the extent to which the factors that mediate such reprogramming operate in a species-specific manner is not understood, the results reported by Dominko et al. show that factors in bovine oocytes are capable of reprogramming the chromatin of differentiated cells of three other mammalian species - sheep, pigs, and monkeys, but not of rats, to generate a nuclear transfer unit that can sustain embryogenesis leading to formation of a "blastocyst-like structure with distinct blastocyst morphology," including an inner cell mass, trophectoderm, and blastocoel cavity (Dominko et al., page 1500).

Early embryogenetic events occur in the absence of transcription of the embryonic genome, and are controlled by maternally inherited gene products in the oocyte cytoplasm. The time during embryogenesis at which transcription of embryonic genes begins is referred to as the "maternal to embryonic" (MET) transition, and occurs at a species-specific developmental stage. For example, transcription begins in bovine and porcine embryos at the late 4- to early 8-cell stage, in murine and rat embryos at the 2- to 4-cell stage, in sheep embryos at the 8- to 16-cell stage, and in human embryos at the 4- to 8-cell stage (see Dominko et al., page 1500, left column; and First et al., International Publication No. WO 99/05266, published Feb. 4, 1999, page 13, copy attached). The dependence of the timing of later embryogenetic events on the species of the nuclear donor cell observed by Dominko et al. and First et al. further demonstrates that the biochemical metabolism within the cells of

the cross-species embryos is the result of species-specific, time-sensitive, interactions and between the genomic DNA and oocyte-derived factors.

The complement of proteins that is initially produced by the embryonic genome also varies according to the species of the embryo, and the initial pattern of embryonic gene expression is thought to be regulated by species-specific factors present in the oocyte cytoplasm (First et al., paragraph bridging pages 13-14). Species-specific incompatibilities between the oocyte-derived mitochondria and proteins expressed by nuclear genes are another possible reason for species-specific differences in the development and differentiation of embryonic cells of embryos produced by cross-species nuclear transfer. Mammalian mitochondrial DNA codes for 13 enzymes that mediate oxidative phosphorylation, 22 tRNAs, and two rRNAs (Smith et al., J. Reprod. Fertil. Suppl. 48:31-43, 1993, abstract attached). Kenyon et al. show that oxidative phosphorylation is impaired in cells with human genomic DNA and mitochondria of orangutan, New World monkeys, or lemurs, but not in cells with human genomic DNA and mitochondria of gorillas or chimpanzees (Proc. Nat. Acad. Sci. U.S.A., 94:9131-9135, 1997, see pages 9132-9133; copy attached). Dominko et al. state that before the usefulness of cross-species nuclear transfer (e.g., to generate cells and tissue for transplantation, etc.) can be evaluated, it is necessary to determine "the extent and faithfulness of nuclear reprogramming," and other compatibilities between the somatic cell's genetic information and factors in the recipient oocyte cytoplasm, including the mitochondria. Dominko et al. conclude with the statement that embryonic cell lines grown from cross-species embryos are expected to be useful for evaluating "long- and short-term effects of mixing of nuclear and cytoplasmic components of various species" (page 1501).

As taught by Dominko et al. and as discussed above, cells of embryos produced by cross-species nuclear transfer are metabolically distinct from normal cells. Studies of the differentiation of cells isolated from embryos produced by cross-species nuclear transfer such as those of the present invention can provide useful information about the extent and faithfulness of chromatin reprogramming in cross-species embryos, and about inter-specific compatibilities between the complement of genome-encoded proteins and organelles and proteins of the ooplasm. Thus, the claimed cells may be used for purposes for which normal cells are unsuited; e.g., for studying the role of mitochondrial genes in reprogramming and early embryonic development as described, for example, at lines 1-3 of page 2, and lines 29-32 of page 28 of the specification. As the claimed man-made cells are distinguishable from naturally-produced cells, the Applicants respectfully request withdrawal of the rejection of claims under 35 U.S.C. §101 as being directed to products of nature.

Regarding Rejection of the Claims Under 35 U.S.C. §112, First Paragraph

Claims 1–35 were rejected under 35 U.S.C. §112, first paragraph, because undue experimentation would have been required by one of skill in the art to (a) produce pluripotent embryonic or stem-like cells having human genomic DNA and non-human mitochondrial DNA as recited in step (iv) of canceled claim 1; (b) induce embryonic or stem-like cells produced by the disclosed method to differentiate into different types of cells; (c) to use embryonic or stem cells having human genomic DNA and non-human mitochondrial DNA that are produced by the disclosed method; or (d) to provide *in vivo* therapy using embryonic or stem cells having genomic DNA of one species and mitochondrial DNA of a different species that are obtained by the disclosed method.

The Applicants respectfully traverse the rejection of the claims as non-enabled under 35 U.S.C. §112, 1st Paragraph, and on the grounds that the specification enables one skilled in the art to make and use the invention as presently claimed without undue experimentation. New claims 36-87 are directed to a method for generating a nuclear transfer unit by cross-species nuclear transfer, using a differentiated mammalian cell as the nuclear donor cell. Prior to the present invention, it had not been shown and it could not have been predicted that a nuclear transfer unit produced by cross-species nuclear transfer using a differentiated mammalian cell as the nuclear donor cell would cleave and develop successfully to the blastocyst stage, or that cell lines could be produced by in vitro culturing of embryonic cells of such a blastocyst. Wolfe et al. disclosed generating blastocysts from cross-species nuclear transfer units made by transferring embryonic goat and bison cells into enucleated bovine oocytes. Wolfe et al. did not teach that their experimental system would work using a differentiated cell as the nuclear donor. At the time the application was filed, it was generally recognized by those skilled in the art that the chromatin of embryonic cells such as the blastomeres used as donor cells by Wolfe et al. is already in an embryonic configuration, and does not require significant reprogramming by the oocyte cytoplasm to be capable of directing embryogenesis. In contrast, the chromosomes of differentiated donor cells are in a non-embryonic state. Those skilled in the art would have known that in order for the DNA of a differentiated donor cell to be able to successfully direct embryonic development, the chromatin of the differentiated donor cell must be reprogrammed to an undifferentiated state by the oocyte cytoplasm, and that in order for this to occur, (i) the factors in the oocyte cytoplasm that activate expression of genomic genes required for embryogenesis must be structurally compatible with the chromatin proteins and DNA sequences of the donor cell chromatin, (ii) enzymes and other proteins encoded by the donor chromatin must be structurally and metabolically compatible with enzymes and other proteins in the cytosol and

organelles such as mitochondria of the oocyte; and (iii) the biochemical events of reprogramming and activation of the donor cell chromatin must occur with synchrony that is conducive to successful blastogenesis.

As disclosed in the present application (for example, at page 12, lines 5-15), humans and bovines are evolutionarily so far removed from each other that one skilled in the art would reasonably have regarded the Applicants' surprising demonstration that a blastocyst can be successfully generated from a nuclear transfer unit produced by transferring a human differentiated cell into a bovine oocyte, and Applicants' successful isolation of a line of ES-like embryonic cells from such a blastocyst, as pretty strong evidence that other cross-species combinations, especially ones between species that are more closely related than human and bovine, would also yield similar results. Since the present application was filed, scientific reports published by the Applicants and by a number of other research groups have confirmed the teaching of the present application that blastocysts can be successfully generated from nuclear transfer units produced by transferring a nucleus of a differentiated cell of one mammalian species into an oocyte of a different mammalian species. For example, the Applicants have reported (Lanza et al., 2000, Cloning, 2(2):79-90, copy attached) that blastocysts were successfully generated following transfer of skin cell nuclei of a gaur (a species of wild cattle) into enucleated bovine oocytes. Moreover, implantation of the blastocysts into recipient cows resulted in birth of a live cross-species calf with the genomic DNA of a gaur, and the mitochondria of a bovine. Kitiyanant et al. describe the successful generation of blastocysts following transfer of nuclei of fetal buffalo fibroblasts into enucleated bovine oocytes (Cloning Stem Cells, 3(3):97-104, 2001, abstract attached). Loi et al. (Nature Biotechnology, 2001, 19:962-964, copy attached) transferred granulosa cell nuclei of the European mouflon, a species of sheep, into enucleated oocytes of domestic sheep, a different species, and successfully generated blastocysts that developed into healthy lambs

after being implanted into ewes. Dayuan et al. describe the successful generation of blastocysts following transfer of somatic cell nuclei of the giant panda into enucleated rabbit oocytes (Science in China, Series C, 42(4):346-353, 1999, copy attached). Dominko et al. show that transfer of somatic, differentiated cell nuclei of sheep, pigs, and monkeys into bovine oocytes leads to embryogenesis resulting in formation of a "blastocyst-like structure with distinct blastocyst morphology," including an inner cell mass, trophectoderm, and blastocoel cavity (Biology of Reproduction 60:1496-1502, 1999; see page 1500). That one skilled in the art would have regarded the claimed invention as being enabled following Applicants' initial discovery is further indicated by the statement by Dominko et al. that embryonic cell lines grown from cross-species embryos such as those of the present invention are expected to be useful for evaluating "long- and short-term effects of mixing of nuclear and cytoplasmic components of various species" (Dominko et al., page 1501). These references demonstrate that those skilled in the art are able to successfully make and use the claimed invention as described in the specification without undue experimentation. Accordingly, the Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Regarding Rejection of the Claims Under 35 U.S.C. §112, Second Paragraph

Claims 1-35 were rejected under 35 U.S.C. §112, second paragraph, because the meanings of the words "embryonic or stem-like cells" and "desired," and the meaning of the abbreviation DMAP, were considered to be indefinite. The rejected claims are canceled, and while the new claims are directed to similar subject matter, they do not include the language that provided the grounds for rejection under 35 U.S.C. §112, second paragraph. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, second paragraph, be withdrawn.

Rejection of Claims Under 35 U.S.C. §102(e) as being anticipated by Tsukamoto et al.

Claims 18–25 were rejected under 35 U.S.C. §102(e) as being anticipated by Tsukamoto et al. (U.S. Pat. No. 5,716,827). which discloses human hematopoietic stem cells, on the ground that the phrase "human embryonic or stem-like cells" in the rejected claims could be construed to include human hematopoietic stem cells. Rejected claims 18–25 are canceled, and while the new claims encompass embryonic cells having human genomic DNA and non-human mitochondrial DNA, they do not include claims that broadly recite human stem-like cells that would include the human hematopoietic stem cells disclosed by Tsukamoto *et al.* Accordingly, Applicants respectfully request that the rejection of claims under 35 U.S.C. §102(e) as being anticipated by Tsukamoto *et al.* be withdrawn.

Rejection of Claims Under 35 U.S.C. §102(a) as being anticipated by Granerus et al.

Claims 18 – 23 were rejected under 35 U.S.C. §102(a) as being anticipated by Granerus et al. (1996), which discloses a human teratoma cell line. Although claims 18-23 are canceled, Applicants address the rejection as it could apply to the new claims. As discussed above, the metabolism of the cells claimed by the new claims is distinct from that of "normal" cells having genomic DNA and mitochondria and other cytoplasmic factors of the same species. Those skilled in the art are able to use the claimed cells in studies to provide useful information about chromatin reprogramming in cross-species embryos and about inter-specific compatibilities between the set of proteins encoded by the genomic DNA, and the organelles and proteins of oocyte cytoplasm. As discussed above, the specification states that it is an object of the invention to provide cells and methods for such studies; whereas such studies are not possible with the teratoma cells disclosed by Granerus et al. Accordingly, the Applicants submit that the claimed cells are distinct from and are not

anticipated by the human teratoma cells disclosed by Granerus et al., and respectfully request that the rejection of claims under 35 U.S.C. §102(a) as being anticipated by Granerus et al. be withdrawn.

Rejection of Claims Under 35 U.S.C. §102(b) as being anticipated by Yamane:

Claims 18–25 were also rejected under 35 U.S.C. §102(b) as being anticipated by Yamane (1987), which discloses primary cultures of human differentiated cells (abstract on page 219). Although claims 18-25 are canceled, Applicants address the rejection as it could apply to the new claims. Applicants submit that the cells isolated from embryos produced by cross-species nuclear transfer of the present invention are distinct from the normal, human differentiated cells disclosed by Yamane. As discussed above, unlike the cells disclosed by Yamane, the claimed cells have genomic DNA of one mammalian species and mitochondria of a different species, and can be studied to provide useful information about the extent and faithfulness of chromatin reprogramming in cross-species embryos, and about inter-specific compatibilities between the set of proteins encoded by the genomic DNA, and proteins and organelles of the oocyte cytoplasm. Accordingly, Applicants submit that the claimed cells are not anticipated by normal human cells, and respectfully request that the rejection of claims under 35 U.S.C. §102(b) as being anticipated by Yamane be withdrawn.

Rejection of Claims Under 35 U.S.C. §103(a) as being unpatentable over Wolfe *et al.*, taken with Collas et al.:

Claims 1–23 were rejected under 35 U.S.C. 103(a) as being unpatentable over Wolfe *et al.* (1990) in view of Collas et al. (1994). Although claims 1-23 are canceled, Applicants address the rejection as it could apply to the new claims.

Wolfe *et al.* show that cross-species nuclear transfer of embryonic nuclei may produce a nuclear transfer unit that can generate a blastocyst, and Collas et al. teach a method of cross-species nuclear transfer using a differentiated donor cell. But prior to the present invention, no one had shown that a blastocyst could be produced cross-species nuclear transfer using a differentiated mammalian cell as the nuclear donor cell, and that lines of embryonic cells could be produced by culturing embryonic cells derived from such a blastocyst.

At the time the invention was made, one of ordinary skill in the art would have recognized that the chromatin of embryonic donor cells such as those used by Wolfe et al. is in an embryonic configuration that does not require significant reprogramming by the oocyte cytoplasm to be capable of directing embryogenesis, whereas the chromosomes of a differentiated donor cell are in a non-embryonic state, and must be reprogrammed in order for successful embryonic development to occur. One of ordinary skill in the art would have known that in order for the DNA of a differentiated donor cell to successfully direct embryogenesis, the chromatin of the donor cell must be reprogrammed by the oocyte cytoplasm, the factors in the oocyte cytoplasm that activate expression of genomic genes required for embryogenesis must be compatible with the chromatin proteins and DNA sequences of the donor cell chromatin, the proteins encoded by the donor chromatin must be metabolically compatible with proteins and organelles such as mitochondria of the oocyte; and the reprogramming and activation of the donor cell chromatin must occur with timing that is conducive to successful blastogenesis, as discussed above in detail, with respect to the rejection under 35 USC 101. In view of these considerations, at the time the invention was made, one of ordinary skill in the art could not have predicted whether or not a nuclear transfer unit produced by cross-species transfer of a nucleus from a differentiated donor cell into an oocyte of a different species could successfully generate a blastocyst. Only after the

Applicants successfully demonstrated the disclosed invention using species so evolutionarily distant as human and bovine could one of skill in the art have predicted that the claimed method could be performed with a reasonable expectation of success. Accordingly, the Applicants submit that the invention could not have been prima facie obvious at the time the application was filed, and respectfully request that the rejection of claims under 35 U.S.C. §103(a) be withdrawn.

All issues raised by the Office Action dated December 19, 2001, have been addressed in this Reply. Accordingly, a Notice of Allowance is next in order. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that she contact the undersigned so that such issues may be addressed expeditiously.

Respectfully submitted,

Date: June 19, 2002

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APPENDIX

The changes made in the specification by the above amendments are shown below:

IN THE SPECIFICATION:

Please replace the paragraph beginning at line 29 of page 5 with the following amended paragraph:

- - [Also , there have been previous attempts to produce cross species NT units (] Wolfe et al. [,] (*Theriogenology*, 33:350 (1990)) describe fusing [. Specifically,] bovine embryonic cells [were fused] with [bison] oocytes of other mammalian species to produce [some] cross species nuclear transfer (NT) units, some of which divided to form embryonic structures possibly having an inner cell mass. No inner cell mass structures were obtained when cattle nuclei were fused into hamster oocytes during NT. Wolfe et al. did not describe attempts to use non-embryonic cells, e.g., [However, embryonic cells, not] adult cells [were used], as donor nuclei in the nuclear transfer procedure. The dogma has been that embryonic cells are more easily reprogrammed than adult cells. This dates back to earlier NT studies in the frog (review by DiBerardino, *Differentiation*, 17:17-30 (1980)). [Also, this study involved very phylogenetically similar animals (cattle nuclei and bison oocytes). By contrast, previously when more diverse species were fused during NT (cattle nuclei into hamster oocytes), no inner cell mass structures were obtained. Further, it has never been previously reported] Wolfe et al. also did not report that the inner cell mass cells from NT units could be used to form an ES cell-like colony that could be propagated. - -

Please replace the paragraph beginning at line 22 of page 19 with the following amended paragraph:

- - The activated NT units then may be cultured in a suitable *in vitro* culture medium until the generation of embryonic or stem-like cells and cell colonies. Culture media suitable for culturing and maturation of embryos are well known in the art. Examples of known media, which may be used for bovine embryo culture and maintenance, include Ham's F-10 + 10% fetal calf serum (FCS), Tissue Culture Medium-199 (TCM-199) + 10% fetal calf serum, Tyrodes-Albumin-Lactate-Pyruvate (TALP), Dulbecco's Phosphate Buffered Saline (PBS), Eagle's and Whitten's media. One of the most common media used for the collection and maturation of oocytes is TCM-199, and 1 to 20% serum supplement including fetal calf serum, newborn serum, estrual cow serum, lamb serum or steer serum. A preferred maintenance medium includes TCM-199 with Earl salts, 10% fetal calf serum, 0.2 [MM Ma] mM Na pyruvate and 50 μg/ml gentamicin sulphate. Any of the above may also involve co-culture with a variety of cell types such as granulosa cells, oviduct cells, BRL cells, [and] uterine cells and STO cells. - -

Please replace the paragraph beginning at line 19 of page 22 with the following amended paragraph:

- - After NT units of the desired size are obtained, the cells are mechanically removed from the [zone] zona and are then used to produce embryonic or stem-like cells and cell lines. This is preferably effected by taking the clump of cells which comprise the NT unit, which typically will contain at least about 50 cells, washing such cells, and plating the cells onto a feeder layer, e.g., irradiated fibroblast cells. Typically, the cells used to obtain the stem-like cells or cell colonies will be obtained from the inner most portion of the cultured NT unit which is preferably at least 50 cells in size. However, NT units of smaller or greater cell

numbers as well as cells from other portions of the NT unit may also be used to obtain ES-like cells and cell colonies. The cells are maintained in the feeder layer in a suitable growth medium, e.g., alpha MEM supplemented with 10% FCS and 0.1 mM beta-mercaptoethanol (Sigma) and L-glutamine. The growth medium is changed as often as necessary to optimize growth, e.g., about every 2-3 days. - -